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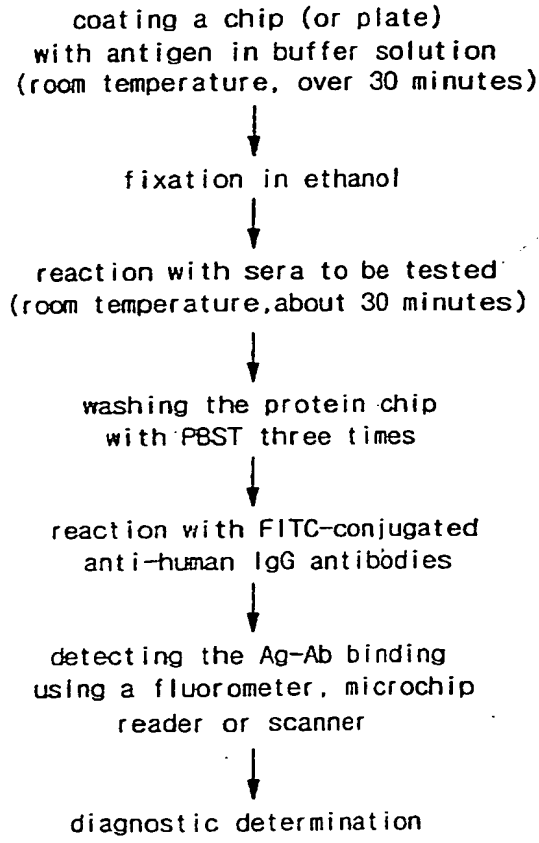
(54) Title: MULTIPURPOSE DIAGNOSTIC SYSTEMS USING PROTEIN CHIPS



(57) Abstract: The present invention provides protein chips on which high density of protein probe arrays are fixed, a method for manufacturing the protein chips, atomized diagnostic systems comprising the protein chips and the use thereof. The highly integrated structure of the protein chip makes a biochemical or an ummunological assay faster, suitable for automatization, precise and easy to handle. The usage of the protein chip encompasses clinical diagnosis, researches for the kinetics of enzymatic reactions and screening antagonists or ligands which bind to the interested receptors. In particular, the protein chip enables multipurpose diagnosis of various diseases for a number of patients even by a test.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

What is claimed is:

1. A protein chip for mass-diagnosis or analysis of test samples, which comprises a micro solid substrate on which a plurality of spots of probe proteins are fixed in a defined arrangement, wherein,

1) the probe proteins are selected from the group consisting of antigens, receptors and enzymes;

2) the probe proteins are fixed on the micro solid substrate via bonds between amino groups of the probe proteins and functional groups of chemicals coated on the substrate;

3) the probe proteins are capable of binding to target proteins in the test samples; and

4) the quantity of the probe proteins per spot is 0.1pg or more.

2. The protein chip of claim 1, wherein the probe proteins are antigenic proteins originated from animals, plants or unicellular organisms including viruses, bacteria and fungi.

3. The protein chip of claim 2, wherein the antigenic proteins originate from Hepatitis B virus (HBV), Human immunodeficiency virus (HIV) or Hepatitis C virus (HCV).

4. The protein chip of claim 1, wherein the micro solid substrate is made of glass, modified silicone or polymer such as polystyrene, tetrafluoroethylene and polypropylene; and, the surface of the substrate is coated with a chemical selected from the group consisting of polymers, plastics, resins, carbohydrates, silica, silica derivatives, carbons, metals, inorganic glasses and membranes.

5. The protein chip of claim 1, wherein the functional group of the chemical coated on the substrate is an alkyl group.

6. The protein chip of claim 5, wherein the chemical coated on the substrate is aminoalkylsilane.

7. The protein chip of claim 1, wherein the spots of the probe proteins are circular in shape and 150~1000㎛ in diameter.

8. The protein chip of claim 1, wherein the quantity of the probe proteins per spot ranges from 1 to 100pg.

9. The protein chip of claim 1, wherein the probe proteins are fixed on the micro solid substrate by the steps of: arraying the probe proteins, previously diluted with a coating buffer, on the substrate in a defined arrangement; immersing the substrate in 100% ethanol; and drying the substrate.

10. The protein chip of claim 9, wherein the coating buffer is sodium phosphate buffer or sodium carbonate buffer.

11. The protein chip of claim 10, wherein the coating buffer is 10mM sodium phosphate buffer or 50mM sodium carbonate buffer.

12. The protein chip of claim 1, wherein the micro solid substrate is a tetragonal plate on which the protein spots are arranged in plural columns and rows or a circular disc plate on which the protein spots are arranged around the circumference.

13. The protein chip of claim 1, wherein the substrate is divided into one or more sectors and each sector has spots containing a same kind of proteins different from those in other sectors.

14. A method for manufacturing the protein chip of claim 1, which comprises the steps of:

1) arraying mixtures of a coating buffer and one or more kinds of probe proteins at predetermined locations on a micro solid substrate, with the quantity of the proteins per spot of 0.1pg or more;

2) immobilizing the probe proteins by incubating the substrate at room temperature;

3) fixing the probe proteins on the substrate by immersing the substrate in 100% ethanol; and

4) drying the substrate obtained in 3).

15. The method of claim 14, wherein the probe proteins of step 1 are selected from the group consisting of antigens, receptors and enzymes which originate from animals, plants or unicellular organisms including viruses, bacteria and fungi.

16. The method of claim 15, wherein the antigens originate from Hepatitis B virus (HBV), Human immunodeficiency virus (HIV) or Hepatitis C virus (HCV).

17. The method of claim 14, wherein the coating buffer of step 1 is sodium phosphate buffer or sodium carbonate buffer.

18. The method of claim 14, wherein the micro solid substrate of step 1 is made of glass, modified silicone or polymer such as polystyrene, tetrafluoroethylene and polypropylene; and, the surface of the substrate is coated with chemicals selected from the group consisting of polymers, plastics, resins, carbohydrates, silicas, silica derivatives, carbons, metals, inorganic glasses and membranes.

19. The method of claim 18, wherein the micro solid substrate is coated

with aminoalkylsilane.

20. The method of claim 14, wherein step 1 contains the steps of dividing the substrate into one or more sectors and arraying the probe proteins on the substrate so that each sector has proteins different from those on other sectors.

21. The method of claim 14, wherein step 1 is performed by an automatic microarrayer system.

22. A method for analyzing target proteins present in test samples quantitatively or qualitatively, which comprises the steps of:

1) reacting a test sample with the protein chip of claim 1 or 12; washing the protein chip;

2) reacting the protein chip obtained in 2) with fluorescence-conjugated secondary antibodies specific for a target protein, said target protein being capable of binding a probe protein fixed on the protein chip; and

3) detecting the reaction signals with a fluorescence microscope or a microchip reader.

23. The method of claim 22, wherein the protein chip has antigenic proteins relating to two or more diseases fixed in divided sectors thereon so that each sector contains proteins different from those on other sectors, the test sample is serum of a subject, and the reaction signals detected with a fluorescence microscope or a microchip reader refer to diagnostic indications for the diseases in the subject.

24. The method of claim 22, wherein the protein chip has antigenic proteins relating to a disease, the test samples are sera of two or more subjects, and the reaction signals detected with a fluorescence microscope

or a microchip reader refer to diagnostic indications for the disease in the subjects.

25. The method of claim 22, wherein the protein chip has antigenic proteins relating to two or more diseases, the test samples are sera of two or more subjects, and the reaction signals detected with a fluorescence microscope or a microchip reader refer to diagnostic indications for the diseases in the subjects.

26. The method of claim 23 or 25, wherein the antigenic proteins relating to two or more diseases are two or more antigenic proteins selected from the group consisting of antigenic proteins of Hepatitis B virus (HBV), Human immunodeficiency virus (HIV) and Hepatitis C virus (HCV).

27. The method of claim 22, wherein the reaction of step 3 is performed by an automatic microarrayer system.

28. The method of claim 22, wherein the fluorescent substance conjugated with the secondary antibodies of step 3 is fluorescein isothiocyanate (FITC).

29. An automated system for diagnosing a plurality of diseases in plural subjects comprising:

1) the protein chip of claim 1;

2) the first microarrayer capable of arraying one or more probe proteins in plural spots on the protein chip;

3) the second microarrayer cotrolled to perform sequentially allotting test samples exactly to the locations at which the probe proteins are fixed on the protein chip, washing the protein chip after reaction, and adding secondary antibodies to react with target proteins in the test samples; and

4) a fluorescence microscope or a micro chip reader for detecting the reaction between the probe proteins and the target proteins.

30. The system of claim 29, further comprising a computerized apparatus which is capable of compiling diagnostic data acquired by the fluorescence microscope or the micro chip reader and processing the data.